DNA Bending by Asymmetric Phosphate Neutralization

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DNA is often bent when complexed with proteins. Understanding the forces responsible for DNA bending would be of fundamental value in exploring the interplay of these macromolecules. A series of experiments was devised to test the hypothesis that proteins with cationic surfaces can induce substantial DNA bending by neutralizing phosphates on one DNA face. Repulsions between phosphates in the remaining anionic helix are predicted to result in an unbalanced compression force acting to deform the DNA toward the protein. This hypothesis is supported by the results of electrophoretic experiments in which DNA spontaneously bends when one helical face is partially modified by incorporation of neutral phosphate analogs. Phasing with respect to a site of intrinsic DNA curvature (hexadeoxyadenylate tract) permits estimation of the electrostatic bend angle, and demonstrates that such modified DNAs are deformed toward the neutralized surface, as predicted. Similar model systems may be useful in exploring the extent to which phosphate neutralization can account for DNA bending by particular proteins.

When studied as a naked polymer in solution, DNA has limited flexibility. DNA stiffness is reflected in estimates of its persistence length [about 140 base pairs (bp)], the distance over which tangents to the initial and final segments of the helix tend to remain aligned (*1*). In contrast, a similar length of DNA (about 145 bp) spontaneously wraps almost twice around an octamer of basic histone proteins, equivalent to a 46° deflection of the DNA for each helical turn (*2*). This remarkable DNA compaction is required to store the approximately 1 meter of human DNA in a nucleus with a diameter of perhaps 10^{-5} m.

Besides the ability of histones to alter the apparent flexibility of DNA, many sequence-specific DNA binding proteins induce significant alterations in the trajectory of the DNA helix. Protein-induced DNA bending is common for eukaryotic transcription factors, including members of the bZIP (*3*) and zinc finger (*4*) families. DNA bending is particularly striking for the TATA element binding protein, TBP (*5*), and the *Escherichia coli* catabolite activator protein (CAP), a protein that bends the DNA in its recognition site by about 90° (*6*). Apart from these transcription factors, other proteins may perform primarily "architectural" roles in influencing the configuration of DNA through nucleoprotein complexes (*7*), and in anchoring DNA loops (*8*). The hypothesis that DNA bending may be a principal function for some of these DNA binding proteins is supported by experiments in which enhancement of recombination or transcription can be achieved by replacing protein binding sites with intrinsically bent DNA sequences (*9*).

Because substantial bending of naked DNA is energetically unfavorable over the short lengths that are typically deformed by bound proteins, the free energy release associated with protein binding must be sufficient to overcome the energetic cost of DNA deformation. We are then faced with the question of how proteins generate this bending force.

It is generally assumed that proteins play an active role in DNA bending and that DNA responds in a passive manner. An alternative hypothesis suggests that DNA plays an active role in the bending process (*10*). According to this hypothesis, DNA possesses latent bending energy stored as electrostatic repulsions between phosphates. When phosphates are neutralized in a laterally asymmetric manner, this source of bending energy is revealed. Counterion condensation theory predicts that cations neutralize (in a thermodynamic sense) a fraction of the negative charge associated with each phosphate diester linkage (*11*). In principle, the residual charge fraction is determined by cation valence, and is relatively insensitive to bulk cation concentration. A substantial contribution to DNA stiffness is predicted to arise from the mutual repulsions exerted between partial negative changes arrayed around the DNA helix. Protein-DNA interactions that result in the formation of salt bridges between cationic amino acid side chains and the phosphate backbone completely neutralize particular phosphate anions, eliminating repulsive interactions with fractional negative charges at neighboring phosphates. The predicted result of the remaining (unbalanced) repulsions is a net bending force causing the DNA double helix to spontaneously relax toward the neutralized surface. Calculations based on this model suggest that the DNA deformation required for nucleosome formation could result from unifacial neutralization of about 10 percent of the total phosphate charge (*10*). Biochemical experiments were designed to test this hypothesis. The results provide evidence that phosphate neutralization, per se, can induce DNA bending.

Measuring electrostatic effects on DNA shape. Many DNA binding proteins present cationic surfaces to DNA (Fig. 1A).

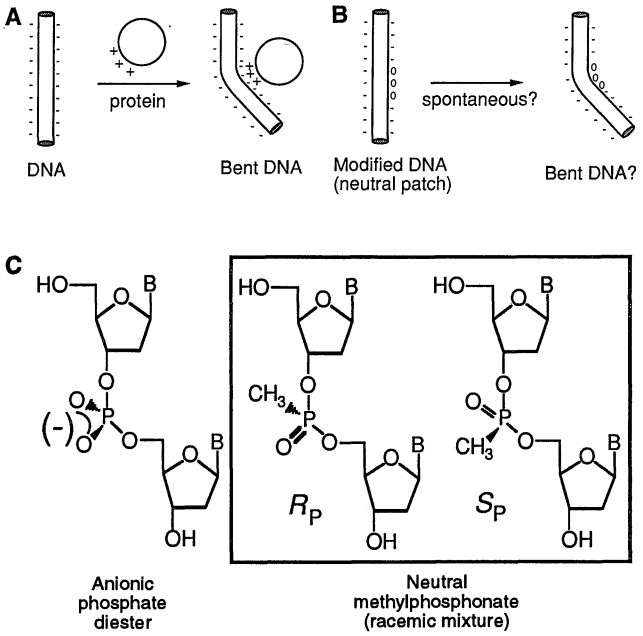

Fig. 1. Experimental model. (**A**) Schematic representation of DNA bending induced by docking to a cationic protein surface. (**B**) Simulation of the electrostatic consequences of protein binding by elimination of a small number of phosphate charges on one face of the DNA helix. (**C**) Neutral methylphosphonates used in these experiments. Racemic mixtures of R_P and S_P isomers were chemically incorporated at each neutralized phosphate position (*26*). Thus, any isomer-specific structural effects are averaged over all substituted positions.

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The extent to which DNA bending results from phosphate neutralization can be determined by turning off phosphate charges on one DNA face, and then measuring the shape of the resulting DNA duplex (Fig. 1B). One strategy for creating such a model requires chemical synthesis of oligonucleotides containing site-specific substitutions of neutral phosphate analogs in place of charged phosphates. The neutral methylphosphonate internucleoside linkage (in which a methyl group is substituted for one of the nonbridging phosphate oxygens) was chosen for this purpose (Fig. 1C). Methylphosphonate analogs were originally proposed for antisense applications (*12, 13*). These analogs have also been used to probe specific protein–nucleic acid contacts (*14*), and the role of phosphate charges in DNA structural transitions (*15*).

DNA curvature can be studied by electrophoretic methods. Mobilities of DNA fragments through polyacrylamide gels exhibit striking shape dependence; for DNA molecules of identical molecular mass, mobility decreases for shapes with reduced end-to-end distances. In a well-studied example, duplex DNAs carrying hexadeoxyadenylate (A_6) tracts appear to be intrinsically curved, and are characterized by anomalously low gel mobilities (*16*). We chose to analyze DNA shape by the phasing method (*17*), wherein an uncharacterized site of helix deformation (the neutral surface) is placed at different positions relative to an internal reference deformation (an A_6 tract) whose magnitude and direction are well characterized (*18*). Synthetic DNA duplexes are ligated to amplify shape effects in the resulting polymers. The net curvature of the DNA duplex reaches extremes as the deformations are phased so as to affect either the same DNA face (cis configuration) or opposite DNA faces (trans configuration). Phasing analysis permits estimation of both the magnitude and direction of the uncharacterized shape.

Synthetic DNA duplexes (21 bp) forming two helical turns of DNA were ligated in a unidirectional orientation. Each 21-bp duplex contained either an A_6 tract alone (known curvature), or an A_6 tract whose center was separated from a patch of six neutralized phosphates by 9.5 bp (cis), 7.5 bp (orthogonal), or 5.5 bp (trans). The neutralized phosphates were positioned across one minor groove (Fig. 2). This configuration was chosen to create a small decrease in overall charge (14 percent) affecting a cluster of phosphates, while not inhibiting enzymatic recognition of molecular termini. The particular DNA sequence was adapted from a previous study in which its helical repeat was shown to be 10.4 ± 0.1 bp per helical turn (*19*).

Bending of DNA toward a neutralized surface. The results of phasing experiments are shown in Fig. 3. Electrophoretic mobilities of ligated duplexes bearing only A_6 tracts (no charge neutralization) are shown in lane 2 of Fig. 3A. When compared with the reference ladder in lane 3, the synthetic duplexes show the anticipated mobility retardation. Lanes 4, 7, and 9, demonstrate that changes in the spacing between an A_6 tract and a patch of charge neutralization cause dramatic changes in gel mobility. This observation shows that a bend is caused by neutralizing one face of the DNA helix. Constructs with the electrostatic bend arranged cis with respect to the A_6 tract curvature display lower electrophoretic mobilities than constructs with A_6 tracts alone (deformations reinforce; compare lanes 4 and 2). As the neutral patch is moved to orthogonal (lane 7) or trans (lane 9) configurations, the gel mobilities of the ladders increase to the point that fragments in lane 9 migrate almost as expected for unmodified linear DNA (deformations tend to cancel). In all cases, the 14 percent charge neutralization has a surprisingly small effect on the mobility of small oligomers (for example, <84 bp). This observation suggests that the electrophoretic effects of charge neutralization on DNA shape are much more important than effects of formal charge per se. From these qualitative results it is possible to conclude that (i) bending of the DNA helix occurs when phosphate charges are neutralized, and (ii) the deformation

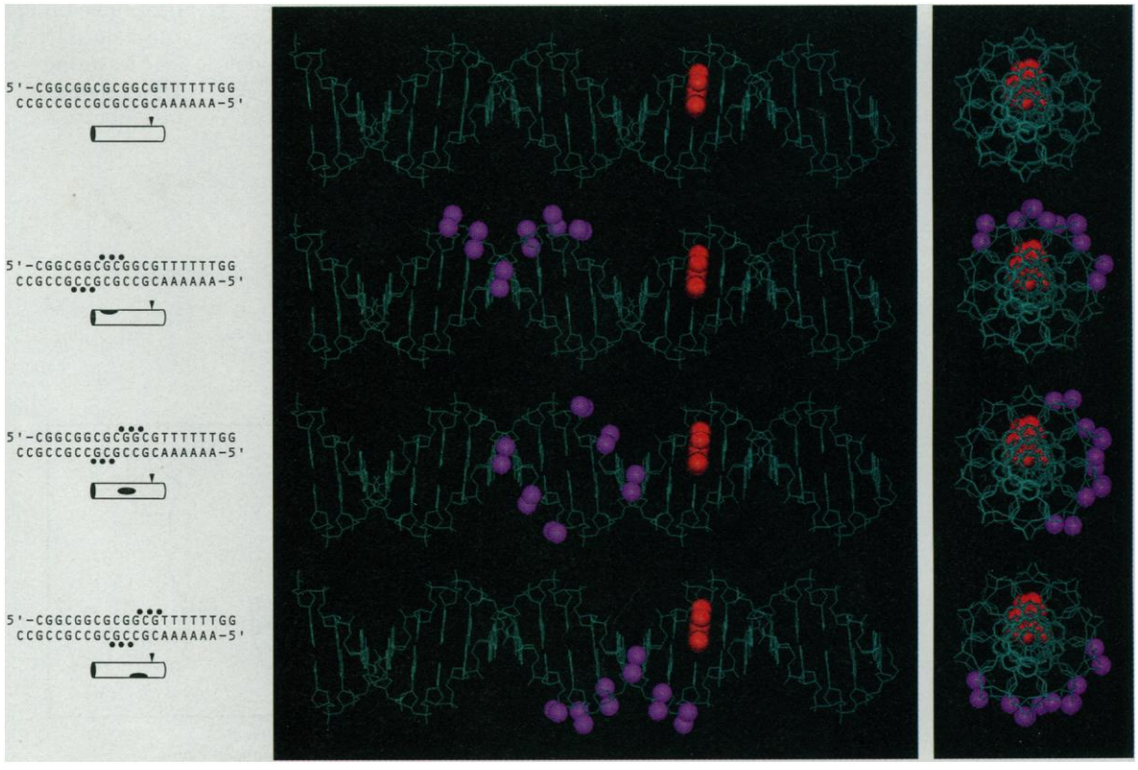

Fig. 2. Design of phasing experiments. Duplex sequences are shown at left (21 bp; two helical turns of DNA). The positions of neutral phosphate analogs are indicated (●). Cylinders depict the spatial relationships between the neutral surface (filled oval) and direction of intrinsic curvature (upward at arrowhead). Molecular models display design details. Atoms comprising the 3′ adenine of the A_6 tract are rendered as red spheres. Duplexes are oriented (in both side and end views) such that the direction of A_6 tract curvature is upward in the plane of the figure (toward the minor groove at the center of the A_6 tract). Neutral phosphates are depicted by rendering both nonbridging phosphate oxygens as magenta spheres (*27*).

bends DNA toward the neutral surface, as predicted.

Electrophoretic data can also yield quantitative estimates of the extent of electrostatic bending (Fig. 3, B and C). Shape information is depicted (Fig. 3B) by plotting the ratio, R_L (ratio of apparent DNA length to actual DNA length), against the actual length of the ligated duplexes. The data were transformed (Fig. 3C) to allow fitting to linear functions relating gel anomaly to the relative curvature for each phasing (*20*). Resulting estimates for net curvatures (A_6 tract equivalents per helical turn) are given at the right of Fig. 3C. By representing helix deformations as vectors (Fig. 4A), data from all three relative phasing arrangements can be combined simultaneously to generate quantitative estimates for the magnitude of the electrostatic bend. A least-squares approach was used to fit data to a phasing equation describing the magnitude of the net curvature as a function of the radial angle between the center of the A_6 tract and the center of the neutral surface (Fig. 4B). The magnitude of the electrostatic bend is estimated to be about 0.58 A_6 tract equivalents per helical turn for neutralization of six phosphates (14 percent charge neutralization overall), distributed across one minor groove. If a value of 18° is used for the helix deformation caused by one A_6 tract, the electrostatic bend amounts to about 21° toward the neutral surface (Table 1). This value may actually represent a lower limit for electrostatic bending, because curvature is directed toward the minor groove in a G·C-rich sequence. Theoretical models suggest that such sequences are particularly resistant to deformation in this direction (*21*).

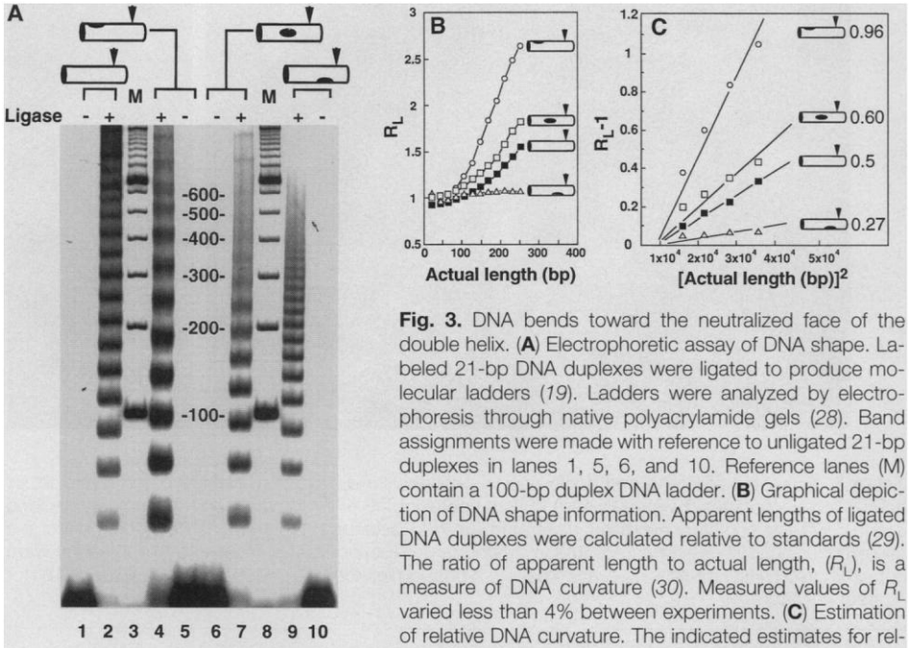

Fig. 3. DNA bends toward the neutralized face of the double helix. (**A**) Electrophoretic assay of DNA shape. Labeled 21-bp DNA duplexes were ligated to produce molecular ladders (*19*). Ladders were analyzed by electrophoresis through native polyacrylamide gels (*28*). Band assignments were made with reference to unligated 21-bp duplexes in lanes 1, 5, 6, and 10. Reference lanes (M) contain a 100-bp duplex DNA ladder. (**B**) Graphical depiction of DNA shape information. Apparent lengths of ligated DNA duplexes were calculated relative to standards (*29*). The ratio of apparent length to actual length, (R_L), is a measure of DNA curvature (*30*). Measured values of R_L varied less than 4% between experiments. (**C**) Estimation of relative DNA curvature. The indicated estimates for relative DNA curvature (average A_6 tract equivalents per helical turn from at least two repetitions) were obtained by fitting data from panel B to an empirical equation relating the observed anomaly in gel mobility to the length and relative curvature of a DNA molecule (*31*). Standard deviations of the DNA curvature estimates for cis, orthogonal, and trans arrangements were 0.05, 0.01, and 0.01 A_6 tract equivalents per helical turn, respectively.

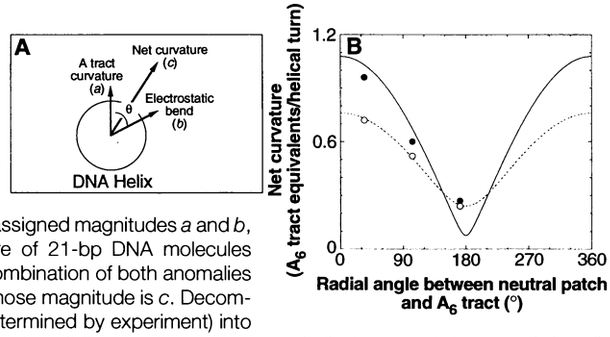

Fig. 4. Interpretation of phasing data. (**A**) Vector description of DNA curvature. The DNA helix is viewed from one end. Static helix deformations due to intrinsic A_6 tract curvature and electrostatic bending (orthogonal to the helix axis and separated by a radial angle, θ) are assigned magnitudes *a* and *b*, respectively. The net curvature of 21-bp DNA molecules affected by the independent combination of both anomalies is given by their vector sum, whose magnitude is *c*. Decomposition of net curvature (*c*; determined by experiment) into components due to A_6 tract curvature (0.5 A_6 tract equivalents per helical turn) and electrostatic bending, allows estimation of the latter. (**B**) Phasing data for two ionic conditions. The plot depicts estimates of the magnitude of net DNA curvature (*c*) as a function of the radial angle (θ) between the A_6 tract curvature and the neutral DNA surface. For example, if two bends of equal magnitude are phased to oppose each other, the net curvature will be zero. In all other cases (where the two bends are of unequal magnitude), the magnitude of the net curvature will oscillate between a maximum value and a minimum value (minimum net curvature > 0) as a function of θ. Filled circles indicate values of net curvature obtained in gels containing about 45 mM tris cation. Solid line shows the least squares fit of the data to an equation describing the phasing experiment (*32*), yielding an estimated electrostatic bend magnitude, *b*, of about 21°. Open circles and corresponding dashed line show data obtained in gels containing about 45 mM tris cation and 1 mM spermidine^{+3}. The latter fit estimates an electrostatic bend magnitude of about 10°. These data reflect averages of at least two experiments. Standard deviations of net curvature estimates were less than 0.05 A_6 tract equivalents per helical turn in all cases.

Distinguishing electrostatic and nonelectrostatic effects. Although dinucleoside methylphosphonates show high structural similarity to the corresponding phosphate diesters (*22*), the analogs are not perfect isosteres (*13, 23*). Three experiments were therefore performed to demonstrate that DNA bending at the site of phosphate neutralization reflects primarily electrostatic (rather than structural) perturbations associated with methylphosphonate substitution.

First, counterion condensation theory predicts that the extent of DNA bending should depend on the fractional residual phosphate charge (a function of counterion valence). Thus, electrophoresis in the presence of di- or trivalent cations is predicted to result in a lower residual negative charge per phosphate (*11*), implying a reduction in compressive force operating on the neutral surface. Reduced electrostatic bending is the anticipated consequence. The experiment shown in Fig. 3 was therefore repeated in the presence of standard buffer (about 45 mM tris cation) supplemented with 1 mM spermidine^{3+} (Fig. 4B, open circles and dashed curve) (Table 1). The estimated magnitude of the electrostatic bend was

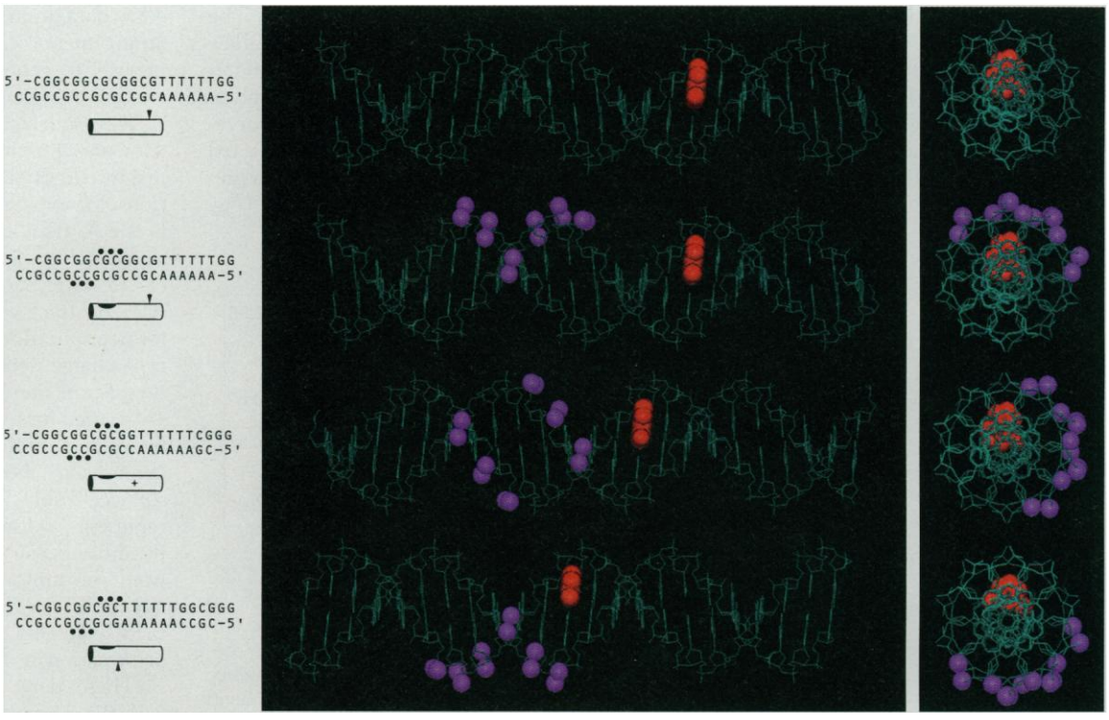

Fig. 5. Design of phasing experiments wherein the position of a sequence-directed DNA curve is varied relative to a stationary array of six neutral phosphate analogs. Duplex sequences are shown at left. The positions of neutral phosphate analogs are indicated (●). Cylinder diagrams and molecular models are interpreted as in Fig. 2. "+" indicates that arrowhead is directed into the page. Duplexes are again oriented (in both side- and end-views) such that the direction of A_6 tract curvature is upward in the plane of the figure.

reduced from about 21° to about 10° in the presence of spermidine^{3+}. Similar effects were observed in the presence of 0.1 mM $Co^{3+}(NH_3)_6$ or 6 mM Mg^{2+} (Table 1). These results show that DNA bending induced by methylphosphonate substitution is due primarily to an electrostatic effect.

Second, it was necessary to determine whether electrostatic bending was influenced by DNA sequence. Sequence-dependence could indicate the involvement of perturbations other than charge neutralization. Because each neutral patch in the initial design involves a different combination of G and C residues, the sequence contexts of the substitutions are not identical and the induced bends could be different. Therefore, an alternative phasing strategy was adopted, in which the position of an A_6 tract was varied relative to a stationary patch of neutral phosphates (Fig. 5). It was also necessary to confirm that unmodified DNA duplexes containing A_6 tracts at any of the three phasing positions were equally bent (Fig. 6A). Phasing of these A_6 tracts relative to a fixed patch of six neutral phosphates provided the data shown in Fig. 6, B and C). Estimates of net values for relative curvature (Fig. 6C) (Table 1) are in agreement with the previous design (Fig. 3). The similarity in these experimental results suggests that bending induced by methylphosphonate substitution is independent of precise sequence context, which is consistent with a predominantly electrostatic effect.

Third, if methylphosphonate substitution introduces unintended changes in helix geometry, these perturbations are likely to affect the helical repeat of the synthetic DNA duplex. Helical repeat can be estimated by varying the length of curved synthetic duplexes that are subsequently ligated together and analyzed by electrophoresis. When the duplex length approximates the helical repeat, helix deformations are coherently phased and the observed mobility anomaly will be maximized. Synthetic duplexes (Fig. 7A) were designed to contain ten methylphosphonate substitutions symmetrically arranged with respect to the helix axis so that no net electrostatic bending is predicted. Any distortions of helix geometry induced by methylphosphonate substitution should affect the helical repeat in an additive manner. Analysis of the relation of gel mobility anomaly (R_L) to duplex length (Fig. 7B) shows that the helical repeat for the highly modified duplexes (about 10.4 bp per helical turn) is comparable to an estimate based on known characteristics (*19*) for unmodified duplexes of the same sequences (about 10.5 bp per helical turn). Thus, there is no evidence that extensive methylphosphonate substitutions alter the helical repeat of these sequences. This result further supports an electrostatic interpretation for the induced bend.

Table 1. Estimates of DNA bend angle due to phosphate neutralization. Neutral patch refers to six neutralized phosphates. Electrophoresis buffers contained 45 mM tris cation and the indicated added cations. Relative curvature estimates were normalized to data for A_6 tract standards studied under the same conditions (*31*). Bend angles (mean ± standard deviation from at least two experiments) were approximated by fitting relative curvature data to a phasing function (*32*), with a constant value of 18° for A_6 tract curvature.

Sequence		Added cations	Estimated neutralization bend (°)
Stationary	Moved		
A_6 tract	Neutral patch	None	20.7 ± 0.3
A_6 tract	Neutral patch	1 mM spermidine^{3+}	9.7 ± 0.4
A_6 tract	Neutral patch	0.1 mM Co^{3+} $(NH_3)_6$	6.0 ± 0.3
A_6 tract	Neutral patch	6 mM Mg^{2+}	6.7 ± 0.3
Neutral patch	A_6 tract	None	23.9 ± 0.8

Implications for DNA bending by proteins. A detectable deformation is induced in a 21-bp DNA duplex by neutralization of a small number of phosphate charges (14 percent) on one helical face. The deformation is interpreted as a static bend of about 20° directed toward the neutral surface (*24*). This observation identifies a general principle that may explain many cases of DNA bending by proteins. For example, it has been suggested that nucleosome formation is accompanied by 10 to 20 percent neutralization of DNA phosphates (*10, 25*). Extrapolation of the electrostatic bending phe-

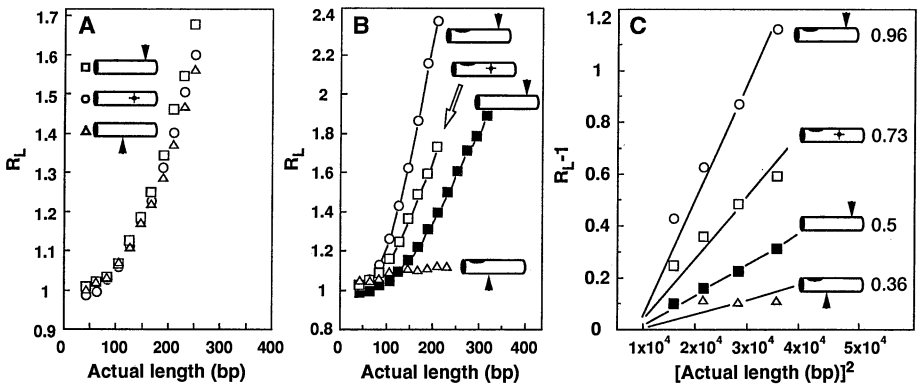

Fig. 6. Confirmation of DNA bending toward the neutralized face of the double helix by phasing A_6 tracts relative to a stationary array of neutral phosphate analogs. (**A**) In the absence of neutralized phosphates, changing the placement of an A_6 tract does not alter DNA curvature. Methods were as described for Fig. 3. (**B**) Graphical depiction of DNA shape information. (**C**) Estimation of relative DNA curvature (average A_6 tract equivalents per helical turn). Standard deviations of the DNA curvature estimates for cis, orthogonal, and trans arrangements were 0.05, 0.03, and 0.03 A_6 tract equivalents per helical turn, respectively.

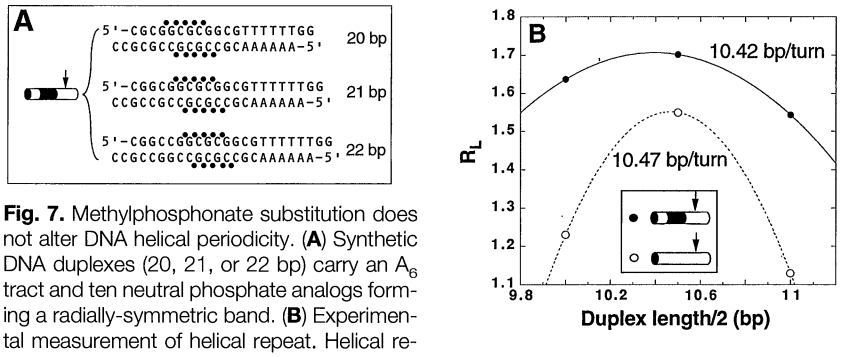

Fig. 7. Methylphosphonate substitution does not alter DNA helical periodicity. (**A**) Synthetic DNA duplexes (20, 21, or 22 bp) carry an A_6 tract and ten neutral phosphate analogs forming a radially-symmetric band. (**B**) Experimental measurement of helical repeat. Helical repeat was estimated as the *y*-axis value at the maximum of a parabolic function fit to the data. Electrophoretic measurements for synthetic duplexes from panel A are indicated (●) in comparison with similar data (*19*) based on the published behavior of the unmodified duplexes (○). Data correspond to average R_L values (from two experiments) extrapolated to a length of 147 bp. Standard deviations of R_L estimates were less than 0.02 in all cases.

nomenon reported here (about 10° electrostatic deflection per helical turn) to the case of nucleosome formation (about 145 bp of DNA; 47° deflection per helical turn) suggests that 14 percent charge neutralization could contribute favorably to DNA bending in nucleosomes. Since the free energy of DNA deformation should increase with the square of the angle of deformation, it is likely that the free energy contribution of phosphate charge neutralization can explain only a portion of the extreme DNA curvature observed in nucleosomes. However, arranging a similar number of neutralized phosphates in different distributions or sequence contexts may permit even greater electrostatic bending. Additional experiments are required to explore this possibility.

Perhaps sequence-specific DNA binding proteins that strongly bend DNA can be categorized with respect to a small number of conserved strategies used for generating force. Proteins such as the TATA-binding protein bend DNA away from the binding site in a manner that appears inconsistent with the electrostatic model that we tested (*5*). The operative strategy in these cases appears to depend on widening the minor groove (*7*). However, many other DNA binding proteins such as *E. coli* CAP deflect DNA toward themselves upon binding. Our results suggest that a substantial fraction of the free energy required for DNA bending in these complexes may be attributed to DNA relaxation toward its neutralized surface. If confirmed, this general principle may provide an approach to understanding the forces that stabilize many nucleoprotein complexes.

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18. Although the exact location (or locations) of helix deformation within an A_6 tract has been the subject of debate, the orientation of the bend is taken to be about 18° toward the minor groove when the helix is viewed from the center of the A_6 tract. Thus, separation of an unknown anomaly by an integral number of helical turns from the center of the A_6 tract will phase both anomalies on the same face of the helix.
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24. Exploration of other interpretations, including the possibility that asymmetric neutralization increases anisotropic flexibility, will require other experimental techniques [D. M. Crothers, J. Drak, J. D. Kahn, S. D. Levene, *Methods Enzymol.* **212**, 3 (1992); J. D. Kahn, E. Yun, D. M. Crothers, *Nature* **368**, 163 (1994)].
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26. Oligodeoxyribonucleotides containing site-specific methylphosphonate substitutions were synthesized at 1 μmol scale with methylphosphonamidite monomers obtained from Glen Research (Sterling, VA). Cytosine residues were protected as isobutyryl derivatives to facilitate cleavage from the solid support and deprotection as described [R. I. Hogrefe, M. M. Vaghefi, M. A. Reynolds, K. M. Young, L. J. Arnold, *Nucleic Acids Res.* **21**, 2031 (1993)]. Oligomers were purified by denaturing polyacrylamide gel electrophoresis, elution from the gel, and desalting with C_{18} reversed phase cartridges; the purified oligomers were characterized by laser desorption mass spectroscopy [U. Pieles, W. Zurcher, M. Schar, H. E. Moser, *ibid.*, p. 3191.
27. Molecular models were generated in SYBYL with a

helical repeat parameter of 10.0 bp per helical turn. Because the helical repeat for these DNA molecules in solution is close to 10.4 bp per helical turn, the images only approximate the relative positions of neutralized phosphates and A_6 tracts for illustrative purposes. All phasing calculations were performed with a helical repeat value of 10.4 bp per helical turn.

28. Samples were analyzed on 5 percent polyacrylamide gels (29:1, acrylamide:bisacrylamide ratio; gel size 15.8 cm by 22.8 cm by 0.75 mm). Casting and running buffers were 90 mM tris borate, EDTA (TBE) unless otherwise noted. Electrophoresis was performed at room temperature (11 V/cm) until the bromophenol blue marker reached 18 cm from the base of the wells. Gels were dried and imaged by storage phosphor technology with a Molecular Dynamics PhosphorImager.
29. The distance migrated by duplex DNA standards of known length was measured and fit by a least-squares method to an exponential function. The apparent length of DNA in each gel band was then estimated from the derived function and the distance migrated.
30. It has been proposed that ratios of gel mobilities (rather than R_L values) provide a better parameter for reporting effects of DNA shape detected by electrophoresis [Y. Hodges-Garcia and P. J. Hagerman, *Biochemistry* **31**, 7595 (1992)]. R_L values were favored in our analysis because of the availability of published data for these oligonucleotide sequences in terms of R_L.
31. An equation of the form

$$R_L - 1 = (pL^2 - q)(\text{relative curvature})^2$$

was fit by a least-squares method to data for duplexes containing one A_6 tract per 21 bp (relative curvature = 0.5 A_6 equivalents per helical turn) and no neutral phosphates. L is the actual DNA length (base pairs). R_L data for 120 bp < duplex length < 190 bp were used for the analysis. This procedure estimates optimum values of the constants p and q for each gel (*20*). Typical values for p and q were $\sim 5 \times 10^{-5}$ and about 0.5, respectively. The resulting equation was used to obtain estimates for unknown relative curvature values for duplexes containing both A_6 tracts and neutralized phosphates. This approach is most accurate for DNA molecules where $R_L > 1.2$.
32. Estimates for the magnitude of the electrostatic bend, b, were calculated by obtaining the least-squares fit of a phasing equation (derived from the trigonometric "law of cosines")

$$c = [a^2 + b^2 - 2ab\cos(180 - \theta)]^{0.5}$$

to plots of the net curvature as a function of radial angle, θ. Constant a is the magnitude of the curvature due to the A_6 tract (0.5 A_6 equivalent per helical turn). Dependent variable c is the measured value of the net curvature (same units), and b is the unknown magnitude of the electrostatic bend (same units). Estimates of net curvature in degrees were obtained using a value of 18° for the deflection of the DNA helix axis by a single A_6 tract (*16, 20*). Note that this phasing equation is unrelated to a trigonometric relation that has been applied to other aspects of DNA bending [J. F. Thompson and A. Landy, *Nucleic Acids Res.* **16**, 9687 (1988)].

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